The influence of a Christmas market on hibernating bats in a man-made limestone cave

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Abstract: It is generally acknowledged that human activity in bat hibernacula can disturb hibernating bats and that such activities need to be appropriately managed. The intensive commercial exploitation of limestone excavations during bats' hibernation period may be in conflict with the micro-environmental conditions that bats need to hibernate. The Fluweelengrot is a limestone quarry in the south of Limburg (the Netherlands). From 1997 onwards it has hosted a Christmas market that attracts over 100,000 people each November and December. The presence of so many visitors and fifty or so illuminated stalls drastically changes the microclimate of part of the cave system for 4-5 weeks. The parts of the cave system occupied by the Christmas market experience a temporary increase in substrate (ceiling) temperatures of 5-8 °C (with a maximum recorded temperature of 20.1 °C). To investigate the possible impact of the Christmas market on hibernating bats in the Fluweelengrot, we examined annual bat census data between the years 1980 and 2010. Data was divided into two periods: before the Christmas market (1980-1997) and during the Christmas market (1998-2010). From 1980 to 2010 seven species were found hibernating in the Fluweelengrot of which five were present in sufficient numbers to calculate trend indices. These were compared with the average indices in 89 other limestone quarries in south Limburg. For the whiskered/Brandt's bat (*Myotis mystacinus* and/or *M. brandtii*) and Geoffroy's bat (*Myotis emarginatus*), the trends in the number of individuals recorded in the Fluweelengrot were significantly less favourable than in other caves in south Limburg. In the absence of changes in other variables this suggests that the Christmas market has a negative impact on these species. Trends in the numbers of recorded pond bats (*Myotis dasycneme*), Daubenton's bats (*Myotis daubentonii*) and Natterer's bats (*Myotis nattereri*) did not differ significantly between the Fluweelengrot and other caves in the area. However a comparison of the distribution of these species of bats before (1990-1997) and during the Christmas market showed a significant shift in their distribution to parts further from the site of the Christmas market. The distribution of the whiskered/Brandt's bat and Geoffroy's bat was similar before and after the start of the Christmas market. Increasing commercial exploitation in a number of the marl caves in the south of Limburg is a cause of concern. Given the major importance of these caves as hibernacula for bats, including three species protected under Annex II of the EU Habitats Directive, we propose that impact assessment studies should be carried out to investigate the potential effects of human activities on hibernating bats in the caves.

Keywords: bats, Chiroptera, cave, quarry, Fluweelengrot, Christmas market, the Netherlands, human disturbance, census, temperature, population trend, distribution.

Introduction

Bat populations are affected by a wide range of stressful influences, one of these being human disturbance of caves that may be used as either summer roosts or as hibernacula. Human disturbance in caves is known to have caused many population declines of cave-dwelling bats (Barbour & Davis 1969, Tuttle 1979, American Society of Mammalogists 1992, Johnson et al. 1998, Wegiel & Wegiel 1998). For this reason, one key aspect of bat conservation is to eliminate or control human entry into caves by, for instance, constructing a gate or fence at the entrance.

The Fluweelengrot is a subterranean limestone quarry in the municipality of Valkenburg in the south of the Province of Limburg, the Netherlands. It is known to have been used by hibernating bats at least since the annual bat censuses started in 1940. Since the middle of the 20th century the Fluweelengrot has also been a tourist attraction. During most of the year, there are daily guided tours of the historical carvings, wall paintings and sculptures found throughout the cave. Since November / December 1997, an annual Christmas market has been organised, running from late November until Christmas. This event attracts thousands of people each year and is thought to considerably disturb the bats that hibernate in the Fluweelengrot. This paper aims to investigate the influence of the Christmas market on the numbers of hibernating bats in the Fluweelengrot, by comparing the population trends of different bat species in the Fluweelengrot to those in similar caves in the south of Limburg, and by investigating the distribution of bats within the Fluweelengrot in relation to the Christmas market.

Hibernating bats frequently arouse to drink, copulate and/or feed. The frequency and purpose of natural arousals varies between and within species; e.g. feeding is uncommon in cave-dwelling bats (Boyles et al. 2006). Arousals from torpor bouts during hibernation are energetically expensive. Naturally occurring arousals from torpor may be responsible for the depletion of as much as 75% of the fat of hibernating bats (Thomas et al. 1990). Arousals may also be provoked by human disturbance. Bats can apparently get used to a low level of human activity during hibernation. However, excessive disturbance, leading to an increased frequency of arousal, may cause bats to abandon a site or decrease winter survival rates. Thus, visits to caves where bats hibernate should be kept to a minimum to reduce the risk of fat depletion caused by unnecessary arousals, which may threaten the survival of the bats (Speakman et al. 1991, Thomas 1995, Mitchell-Jones et al. 2007, Boyles & Brack 2009).

The behavioural response of hibernating bats to disturbance is poorly documented. Thomas (1995) reported a significant increase in bat activity in a hibernation site in the hours following a visit, in spite of no bats being handled. This indicates that bats can be influenced by non-tactile disturbances even if they do not arouse immediately. The sensitivity of bats to human disturbance is also shown by several cases of recovery of bat populations (including species of *Myotis*, *Plecotus* and *Rhinolophus*) after human access to hibernation sites was reduced and / or bat banding ceased (Daan et al. 1982, Voûte & Lina 1986, Gaisler & Chytil 2002, Grol & Voûte 2010).

Human visitors to caves and underground quarries may impose different non-tactile stimuli to hibernating bats, such as light, sound and heat. Thomas (1995) assumed light and sound to be responsible for the increased flight activity detected after human visits. The Christmas market in the Fluweelengrot brings sound, light and heat to the cave, produced by the presence of thousands of visitors and by the power units used for lighting the cave. This is likely to change the microclimate of the Fluweelengrot, decreasing the humidity and increasing the ambient temperature.

Harmful effects of high ambient and substrate temperatures at roosts of hibernating bats have been reported by Humphrey (1978), Richter et al. (1993) and Martin et al. (2006). A microclimatic change in a hibernaculum may cause bats to arouse or lead to changes in the distribution of bats within a cave (Richter et al. 1993), and these responses may differ among species. Changes in the temperature may directly or indirectly act as stimuli to spontaneous arousals (Daan 1973). In an experimental setting Davis and Reite (1967) performed a stepwise increase in ambient temperature, between 5 °C and 10 °C they detected no arousals, but when the temperature was increased to 15 °C, four out of five species studied responded by arousal from dormancy. Brenner (1974) did a controlled experiment with single hibernating bats (Indiana myotis *Myotis sodalis* and little brown myotis *Myotis lucifugus*) and found that Indiana myotis was aroused, and became active, at lower temperatures than little brown myotis. Such differences between species are reflected in the specific temperature zones that different species select for hibernation (Bezem et al. 1964, Daan 1973, Raesly & Gates 1987, Nagel & Nagel 1991, Brack 2007).

Methods

Description of the site

The Fluweelengrot, also known as "Historische Grot" (Bels 1952, van Wijngaarden 1967), is a medium-sized, subterranean limestone excavation situated in the hills bordering the valley of the river Geul in the municipality of Valkenburg aan de Geul, in the Province of Limburg, the Netherlands. The geology of this part of the Netherlands consists of Cretaceous limestone, which is generally soft, with occasional layers of a more solid consistency. The Fluweelengrot is connected to several escape corridors, one of which leads to Valkenburg Castle (figure 1). Bats can enter the Fluweelengrot through several entrances. The tunnel (T in figure 1) is blocked by a brick wall in which a small hole was made to enable bats to fly in and out. The remains of the twelfth century Valkenburg Castle have been a tourist attraction since 1863. During restoration in 1937 a secret underground passage, hewn out of the rock beneath the castle, was discovered. This escape route gave direct access to the Fluweelengrot, which has a corridor system that is nearly 180 metres wide and 230 metres long and is practically horizontal (figure 2). The corridors of this cave are quite uniform in their height and width, both of which average around three metres. In 2010 a new emergency exit was created near tunnel T. Due to their importance for hibernating bats, many of the marl excavations in south Limburg are listed as belonging to Natura 2000 sites. The Fluweelengrot is part of the 'Geuldal' Natura 2000 site.

Human activities in the Fluweelengrot

Ever since guided tours started to take place in the 1940s, the Fluweelengrot has a regular (almost) year-round flow of tourists, mainly visiting its wall paintings and sculptures. The number of visits per day varies over the year, but on busy days in spring, summer and autumn between 11 a.m. - 4.30 p.m., guided tours of approximately 50 minutes can start every half an hour, with a maximum of 40 people. In January, the cave is closed to the public, while employees prepare the cave for the season. In February, there are limited numbers of guided tours at 11 a.m. and 1 p.m. only. Guides carry a kerosene lamp.

For several decades the Fluweelengrot has also been used for recreational activities such as marlstone carving, climbing and 'abseiling', sporting events and weddings (there is a chapel) and as a location for television programmes. These activities mainly take place in spring, summer and autumn and less frequently in winter.

Since November/December 1997, an annual Christmas market has been held in the Fluweelengrot, lasting for about five weeks until the 23rd of December. This event attracts thousands of people each year. Visitors follow a circular route which has some fifty stalls, mostly set up in niches in the cave architecture (figure 2). The market is open from noon (10 a.m. at weekends) until 9.30 p.m. During the market, the greater part of the cave is illuminated and heated artificially using generators and electrical heating. During opening hours, the main entrance of the cave is blocked by two rows of plastic curtains to keep the warm and dry air inside. These curtains also block the main entrance for bats.

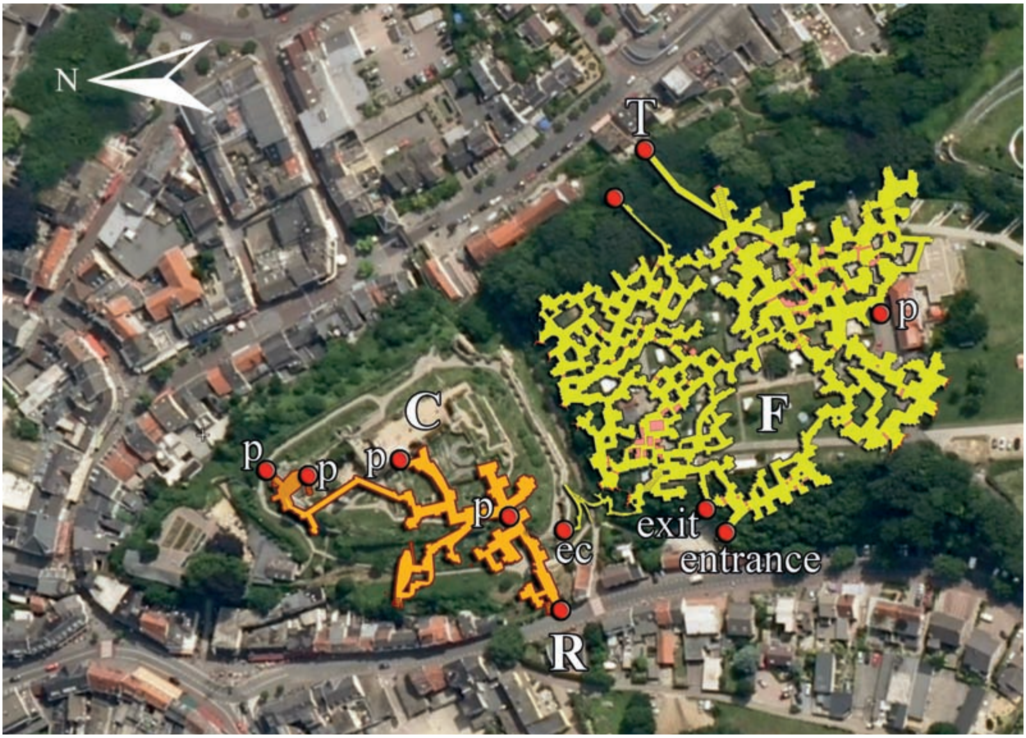


Figure 1. Aerial views of the Fluweelengrot (yellow) and the nearby 'Groeve Onder de Ruïne' (orange) cave systems. F = entrance of the Fluweelengrot, R = entrance of 'Onder de Ruïne' (under the ruin), C = ruins of Valkenburg Castle, p = shaft, T = tunnel, ec = escape route from the castle to the Fluweelengrot. Red dots indicate exit sites. Photo courtesy of Stevenhagen Geo Informatica.

The number of visitors of the Christmas market has increased from 95,000 in 1997 to a maximum of 128,000 in 2007 with an annual mean of 113,000. There has also been a sharp increase in the entrance fee over the years (from € 0.45 in 1997 to € 4.50 in 2011). As such the event has a considerable social and financial impact.

Survey methods

Bat counts have been carried out in the Fluweelengrot almost annually from 1940 onwards. To investigate the possible influences of the Christmas market on hibernating bats, the census data were divided into two periods: from 1980-1997, before the Christmas market began (BCM) and from 1998-2010, during the years of the Christmas market (DCM). 1980 was taken as the start of the period BCM, because bat censuses were standardised at this time, enabling comparison of the counts between years. Every year the bat counts were carried out on January the 2nd or 3rd, when hibernating populations are assumed to be fairly constant (Daan 1973). The one exception was in 2010 when permission to do the census was granted for January 31 because the cleaning of the cave and removal of the Christmas decorations took place early in January. The counts were done with approximately the same group of people using the same method, systematically searching all accessible corridors for bats, using torches and binoculars. The exact location of each bat found was noted on a map, enabling a comparison of their distribution in

Figure 2. Map of the Fluweelengrot with the route of the Christmas market (red line). T = tunnel; bars and grey areas indicate the location of the Christmas stalls; B = pub. Courtesy of Stevenhagen Geo Informatica.

the cave in the BCM and DCM periods. Identification was done without handling the bats. Whiskered bats (*Myotis mystacinus*) and/or Brandt's bats (*Myotis brandtii*) were not distinguished, because of the difficulty in distinguishing between the two species in the field (e.g. Hanák 1970, Hoogenboezem 1982, Dietz & von Helversen 2004). For similar reasons, no distinction is made between brown long-eared bat (*Plecotus auritus*) and grey long-eared bat (*Plecotus austriacus*); both species are simply classified as 'long-eared bat (*Plecotus auritus/austriacus*)'. Bats that, for various reasons, could not be correctly identified were noted as 'species unknown'.

To identify any possible shifts in the bats' positions in the Fluweelengrot in relation to the Christmas market, we compared the distribution during the period 1990-1997 and the DCM period of five (groups of) species: pond bat (*Myotis dasycneme*), the whiskered/Brandt's bat, Natterer's bat (*Myotis nattereri*), Geoffroy's bat (*Myotis emarginatus*) and Daubenton's bat (*Myotis daubentonii*).

Temperature measurements

Substrate temperatures of the ceiling in the Fluweelengrot were measured during a Christmas market (22 December 2009) and eleven days and five weeks after a Christmas market (3 January 2010 and 31 January 2010 respectively) (table 1). We used ceiling temperatures since most bats hibernate near the ceiling in the upper stratified air layers of the cave (de Wilde & van Nieuwenhoven 1954). It was not possible to take measurements outside the Christmas market area while it was in progress in December 2009. For comparison, we included ceiling temperatures taken during the census in 1990 (from just the southern half of the cave), seven years before the first Christmas market was organised (J.P. Bekker, unpublished data). Temperatures were measured at the same locations on all four dates, but fewer measurements were taken on 3 January 1990 (n=69; samples taken in the southern part of the cave only) and 22 December 2009 (n=62; parts of the quarry were inaccessible) than on 3 and 31 January 2010 (n=118).

The temperature samples from 1990 were read from a digital multipurpose thermometer, fixed on a bamboo stick and reaching up to 3 m; samples in 2009 and 2010 were taken with a Fluke 62 Mini Infrared Thermometer (Fluke Corporation, Everett, WA, USA).

Trend analysis

Trend analyses were performed by Poisson regression, using the TRIM programme (TRends and Indices for Monitoring data; version 3.53; Pannekoek & van Strien 2001). Poisson regression was used to analyse count data, because the data was not normally distributed. TRIM computes whether a species has increased or decreased significantly,

Table 1. Ceiling temperature samples taken in (parts of) the Fluweelengrot in 1990, 2009 and 2010, compared with outside daily mean temperature (°C). External temperatures obtained from the Royal Netherlands Meteorological Institute (KNMI). [1] samples in a part of the quarry only.

		Ceiling temp. (°C) Fluweelengrot			Outside temp. (°C) Maastricht		
Date	*n*	Minimum	Mean ±sd	Maximum	Minimum	Mean	Maximum
3 January 1990 [1]	67	2.2	8.6 ± 1.9	10.4	-2.3	0.9	2.9
22 December 2009 [1]	62	5.8	11.5 ± 5.3	20.1	0.2	1.4	2.6
3 January 2010	118	-4.2	9.8 ± 4.3	14.8	-6.7	3.9	0.8
31 January 2010	118	-2.2	7.6 ± 4.0	12.0	-1.5	0.3	1.7

remained stable or, if the change is uncertain. A trend of >1 denotes an increase and a trend of <1 denotes a decrease. We computed the trends before and after November/December 1997 (the start of Christmas market) and tested whether these differed between the Fluweelengrot and the other caves in south Limburg (*n*=89).

Indices were estimated for pond bats, whiskered/Brandt's bats, Daubenton's bats, Natterer's bats and Geoffroy's bats. As only small numbers of long-eared bat and greater mouse-eared bat (*Myotis myotis*) were found, reliable trends could not be established for these two species. Indices started from 1980, except for Natterer's bat and Geoffroy's bat, for which the index started in 1981 (as no Natterer's bats and Geoffroy's bats were found in the Fluweelengrot in 1980).

For temperature data and distribution of the bats, we used two-sided Student t-tests with a significance level of α=0.05.

Results

Bat species and numbers

In the two periods (BCM and DCM) seven species were recorded. Daubenton's bat was the most abundant species in both periods with a yearly average of 32.1 (range 15-51) in the period BCM and 25.8 (range 16-39) in the period DCM (table 2; figure 3a).

The greater mouse-eared bat was the least frequently recorded species. Only single individuals were found in three years (1990, 1991 and 1996) in period BCM, and two and three individuals in 1998 and 1999 (period DCM). The number of long-eared bats was also low in both periods, with a maximum of two records per year over the whole period except for 2008 (DCM), when four long-eared bats were counted. In the period BCM no records were observed in six years, while in period DCM there were three years without records (table 2).

Pond bats were also only found in small numbers in both periods, with a yearly average of 3.7 in the period BCM (range 1-7) and 2.8 (range 0-8) in the period DCM (table 2; figure 3b). Numbers of the whiskered/Brandt's bat fluctuated in both periods, in the BCM period the average yearly number found was 5.9, with a range of 1-14. In the DCM period the yearly average was 5.6, with a range of 0-10 (table 2; figure 3c). 2003 was the only year when no specimens were found. Natterer's bat was recorded in eleven of the 18 census years in period BCM (average per year 1.6, range: 0-7). In period DCM, increasing numbers of this species were found, with an average of 10.4 bats per year and a range of 1-36 records in 2010 (table 2; figure 3d).

A similar trend was found for Geoffroy's bat. This species was either not recorded in the Fluweelengrot, or only in small numbers until the early 1990s, although the numbers increased towards the end of period BCM (average per year 2.9, range 0-12; table 2; figure 3e). In the DCM period, this species was recorded every year, with numbers varying from 5-23 and averaging 11.3 per year.

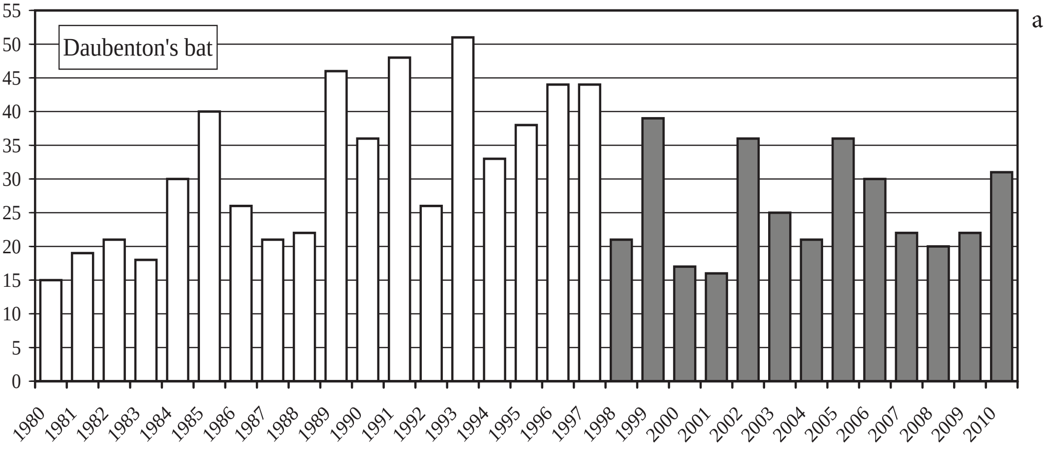
Table 2. Number of hibernating bats found in the Fluweelengrot in the BCM (1980-1997) and DCM (1998-2010) periods. Mmyo = greater mouse-eared bat, Plec = long-eared bat, Mnat = Natterer's bat, Mema = Geoffroy's bat, Mdas = pond bat, Mm/br = whiskered/Brandt's bat, Mdau = Daubenton's bat, indet = species unknown. Sum = total number of bats per year.

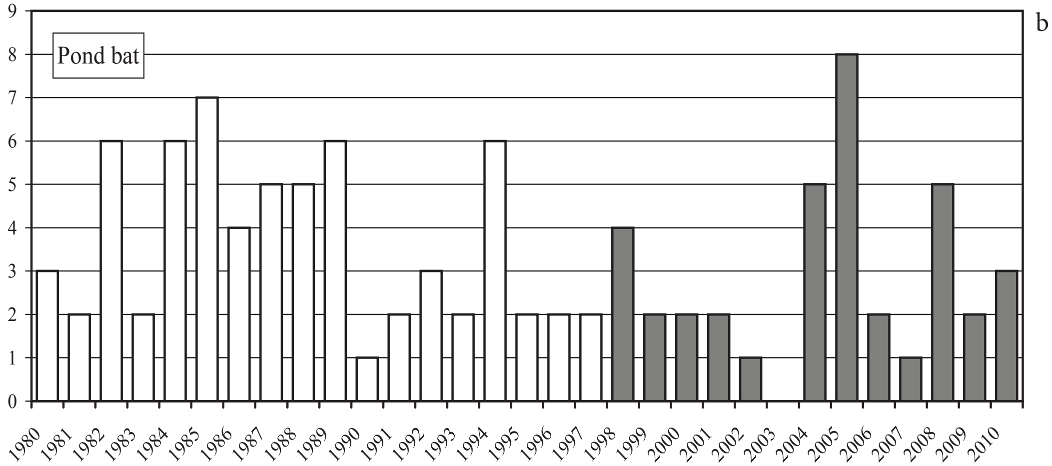
	Species								
Year	Mmyo	Plec	Mdas	Mm/br	Mdau	Mnat	Mema	indet	Sum
1980	0	1	3	1	15	0	0	5	25
1981	0	1	2	3	19	2	3	1	31
1982	0	2	6	3	21	0	0	2	34
1983	0	1	2	6	18	1	0	1	29
1984	0	0	6	7	30	0	0	2	45
1985	0	1	7	3	40	0	1	3	55
1986	0	2	4	13	26	0	0	3	48
1987	0	0	5	7	21	0	1	1	35
1988	0	0	5	6	22	0	2	4	39
1989	0	2	6	2	46	1	2	4	63
1990	1	0	1	14	36	3	0	4	59
1991	1	2	2	8	48	1	3	1	66
1992	0	1	3	3	26	2	2	4	41
1993	0	2	2	4	51	3	3	3	68
1994	0	2	6	6	33	5	6	6	64
1995	0	1	2	11	38	7	10	2	71
1996	1	0	2	4	44	2	7	1	61
1997	0	0	2	5	44	2	12	4	69
1998	2	0	4	9	21	7	12	1	56
1999	3	2	2	4	39	2	11	0	63
2000	0	1	2	5	17	6	7	0	38
2001	0	1	2	9	16	1	5	1	35
2002	0	1	1	8	36	14	12	3	75
2003	0	2	0	0	25	2	5	2	36
2004	0	1	5	6	21	5	10	0	48
2005	0	0	8	8	36	9	8	1	70
2006	0	2	2	3	30	13	19	2	71
2007	0	1	1	6	22	9	11	4	54
2008	0	4	5	2	20	12	7	2	52
2009	0	0	2	3	22	19	17	10	73
2010	0	1	3	10	31	36	23	3	107

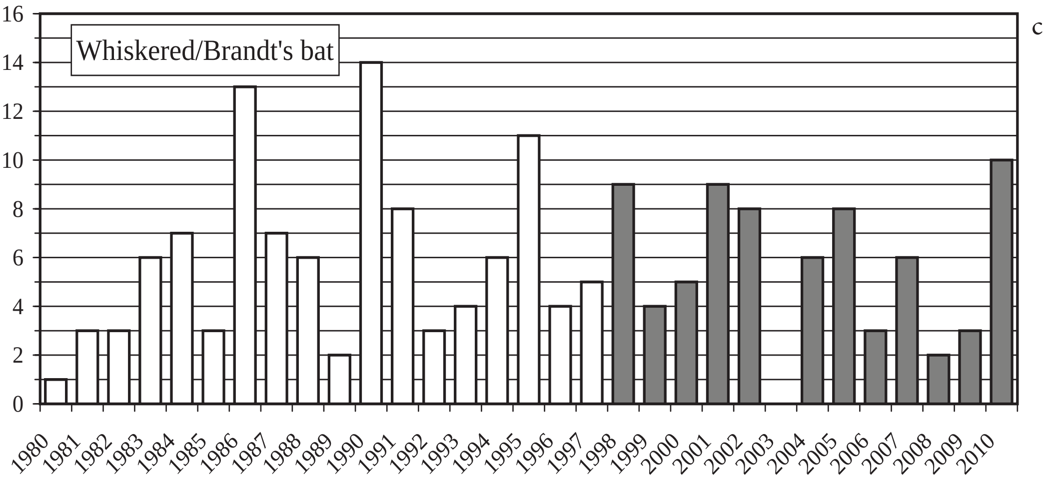
Trends and indices

We used TRIM to compute trends for five bat species and compared indices in the Fluweelengrot to those in the other marl quarries in south Limburg (n=89). For whiskered/Brandt's bat and Geoffroy's bat, there was a significant difference, with the increase in the numbers of both species being less in the Fluweelengrot than in other caves (table 3).

Before the Christmas market the whiskered/Brandt's bat in the Fluweelengrot showed an


Daubenton's bat
a


Pond bat
b


Whiskered/Brandt's bat
c

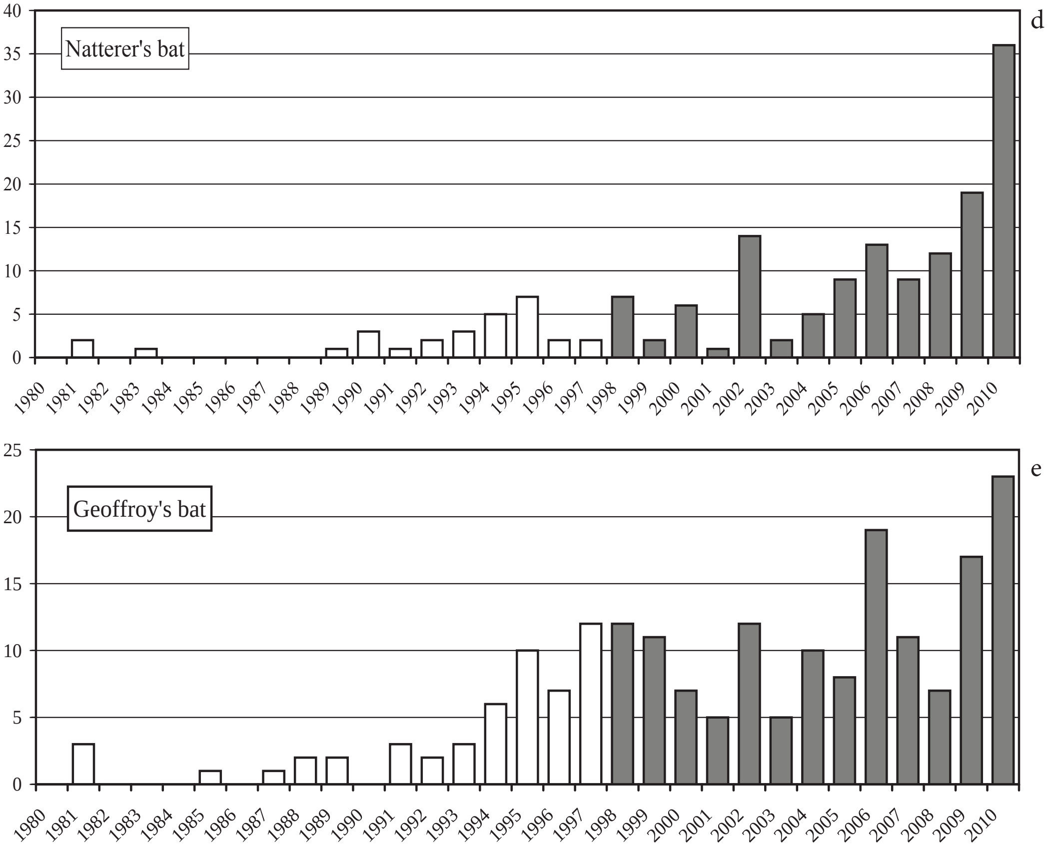


Figure 3. Number of Daubenton's bats (a), pond bats (b), whiskered/Brandt's bats (c), Natterer's bats (d) and Geoffroy's bats (e) from 1980 to 2010, before (white bars) and after (grey bars) the start of the Christmas market.

average annual increase of 3%, compared to a 5% increase in other caves (table 3). In the years after the first Christmas market took place (DCM), the index in the other south Limburg caves increased by 3% per year, while in the Fluweelengrot a decrease of 3% per year was found. Over the whole period, this resulted in a significantly smaller increase of the species in the Fluweelengrot (P=0.02) (table 3; figure 4a).

In the BCM period, the index of Geoffroy's bat in the Fluweelengrot showed a spectacular average increase of 21% per year: this compared to an average of 11% in the other south Limburg caves (table 3). In the DCM period, the increase in the Fluweelengrot was much smaller (4% per year), while numbers in the other caves continued to grow at an average of 13% per year. Taking both periods together, the increase of Geoffroy's bats was significantly lower in the Fluweelengrot than in other caves (P=0.007) (table 3; figure 4b).

For the other three species, the trends in the Fluweelengrot did not differ from those in other caves. Daubenton's bat showed positive trends in the BCM period in both the Fluweelengrot (average annual increase 4%) and in the other south Limburg marl quarries (2%) (table 3; figure 4c), and negative trends for both in the DCM period (average annual decrease 5% and 1% respectively; table 3). The total decrease over both periods in the Fluweelengrot was not significantly different from that in the other south Limburg caves (P=0.10).

Table 3. Trend indices of the pond bat, whiskered/Brandt's bat, Daubenton's bat, Natterer's bat and Geoffroy's bat found in the Fluweelengrot and in other caves in south Limburg in the BCM (1980-1997) and DCM (1998-2010) periods, and probability *P* of differences between the indices over both periods in the Fluweelengrot and in other south Limburg limestone quarries (last column; Poisson regression).

	Period BCM		Period DCM		
Species	Fluweelengrot	Other caves (*n*=89)	Fluweelengrot	Other caves (*n*=89)	$P_{BCM+DCM}$
Daubenton's bat	1.04	1.02	0.95	0.99	0.10
Pond bat	0.97	0.99	1.00	1.04	0.21
Whiskered/Brandt's bat	1.03	1.05	0.97	1.03	0.02*
Natterer's bat	1.13	1.19	1.16	1.16	0.55
Geoffroy's bat	1.21	1.11	1.04	1.13	0.007**

For pond bat and Natterer's bat the trends over both periods, taken together, were similar in the Fluweelengrot and other caves (*P*=0.21 and 0.55 respectively) (figures 4c and 4b). Natterer's bat showed large increases (13-19%) in both periods in both the Fluweelengrot and the other caves in south Limburg.

Temperatures

The temperatures measured at four sampling dates in 1990 (3 Jan), 2009 (22 Dec) and 2010 (3 and 31 Jan) (table 1) were used to develop gradient maps, roughly showing the distribution of ceiling temperatures in the areas sampled (figures 5a-d). On 31 January 2010, 39 days after the Christmas market, a clear temperature gradient was visible in the cave, with temperatures increasing from below 0 °C in the entrance areas in the west and northwest to 10-12 °C in the southeastern part (figure 5d). During the Christmas market, on 22 December 2009, the ceiling temperatures in the area sampled were 5-8 degrees higher (at one location, where a pub was situated, a maximum of 20.1° C was measured) (figure 5b). Nine days after the Christmas market, on 3 January 2010, the figure is somewhere between the two, showing that the quarry was cooling down in the weeks after the Christmas market (figure 5c). Temperatures on 31 January 2010 were similar to those in January 1990, except for the western part of the quarry, where temperatures were lower in 2010, probably due to the long cold winter period (figures 5a and 5d).

Bat distributions within the quarry

A grid overlay was used to calculate the average location of different bat species in the Fluweelengrot over two periods: 1990-1997 and DCM (figure 6). The average location of three species of bat differed significantly between the two periods: pond bats (1990-1997: x ± sd = 42.76 ± 27.42, y= 79.82 ± 21.05, *n*=20; DCM: x = 33.89 ± 21.83, y = 61.21 ± 30.10, *n*=37) (Student's *t*-test, *P*=0.02), Daubenton's bats (1990-1997: x = 39.46 ± 24.82, y = 61.94 ± 24.48, *n*=320; DCM: x = 29.93 ± 19.94, y = 56.66 ± 24.00, *n*=336) (*P*<0.001) and Natterer's bat (1990-1997: x = 36.16 ± 21.40, y= 55.60 ± 26.29, *n*=25; DCM: x = 29.10 ± 17.47, y = 48.51 ± 27.26, *n*=135) (*P*=0.04). These three species showed a shift towards the northwest of the quarry, away from the area most affected by the Christmas market (figures 6a-c). For whiskered/Brandt's bat and Geoffroy's bat, we found no difference between the two periods (whiskered/Brandt's bat: 1990-1997: x ± sd = 31.67 ± 21.19, y= 58.21 ± 27.11, *n*=55; DCM: x = 29.09 ± 18.29, y = 55.67 ± 19.28, *n*=73; Geoffroy's bat: 1990-1997: x = 41.81 ± 19.13, y= 79.82 ± 22.13, *n*=43; DCM: x = 41.21 ± 17.28, y = 67.93 ± 25.87, *n*=147) (*P*>0.05) (figures 6d-e).

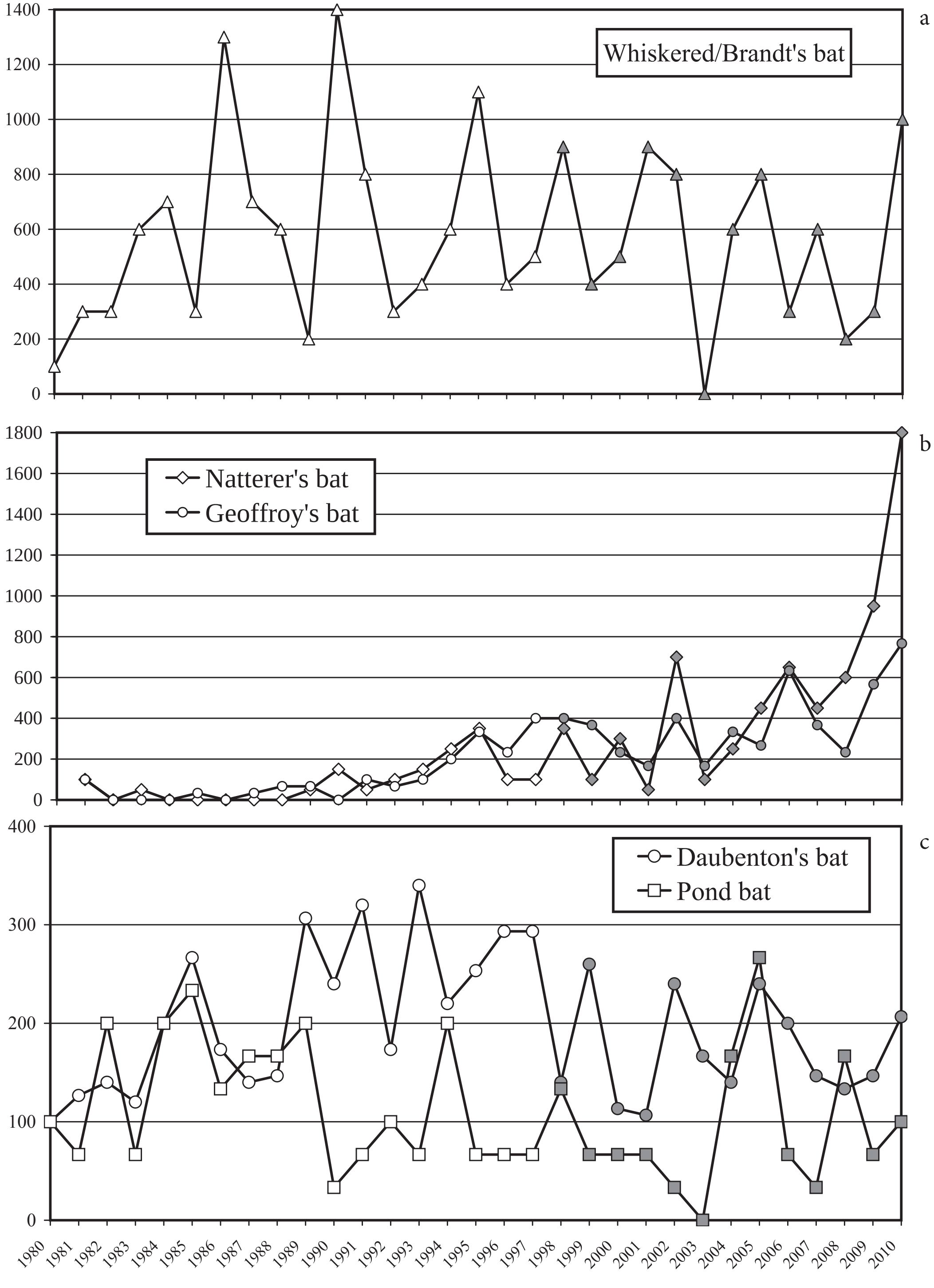
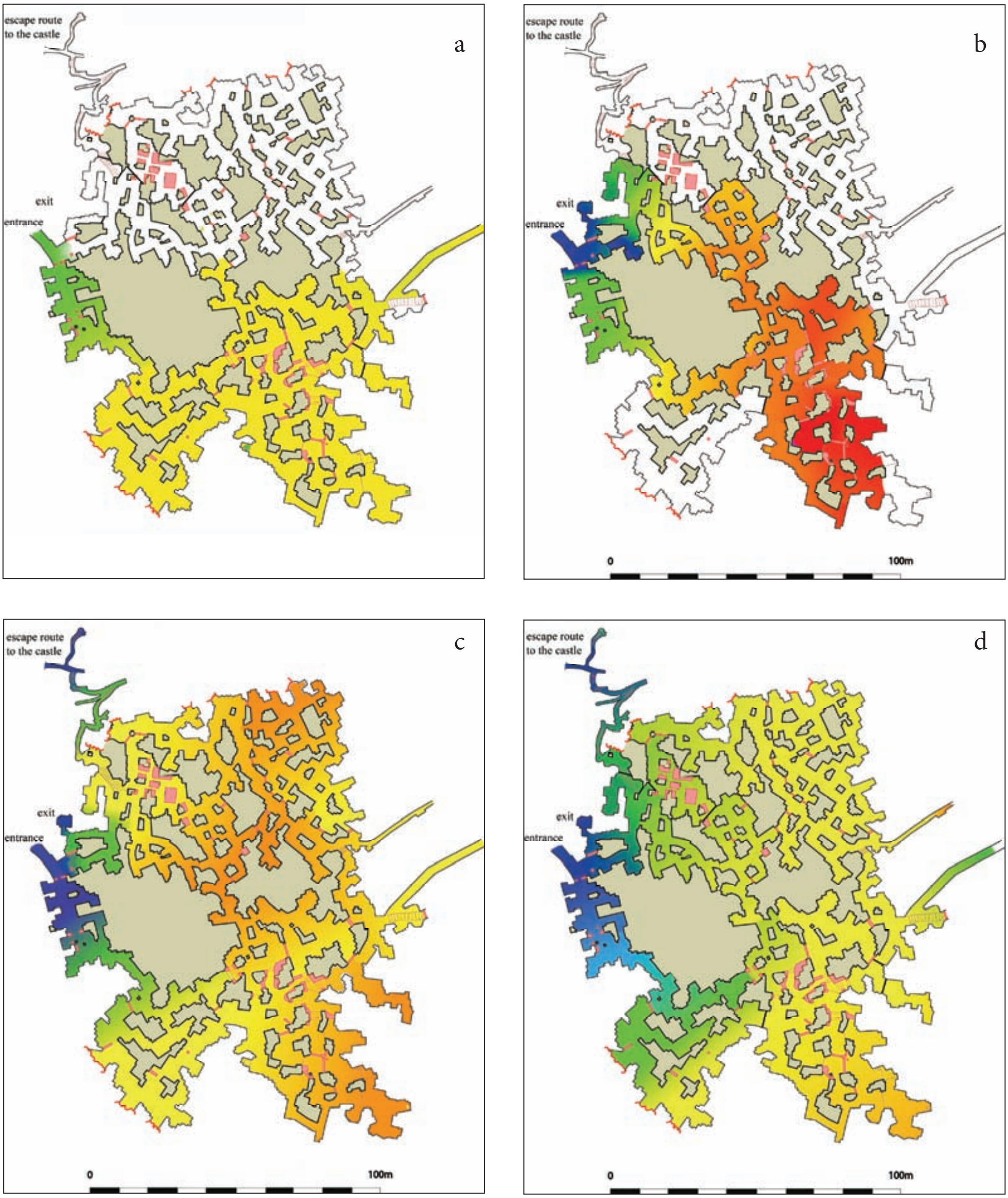


Figure 4. Trend indices of whiskered/Brandt's bat (a), Natterer's bat and Geoffroy's bat (b), and Daubenton's bat and pond bat (c) from 1980-2010. Dark grey points are years with a Christmas market.



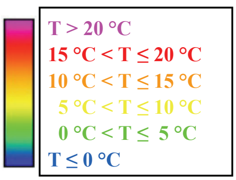


Figure 5. Gradient map of substrate temperatures of the ceiling of the Fluweelengrot on 3 January 1990 (a), 22 December 2009 (b), 3 January 2010 (c) and 31 January 2010 (d). White parts were not accessible during the Christmas market (22 December 2009) or data were unavailable (3 January 1990).

Discussion

We investigated the presumed disturbance to hibernating bats in the Fluweelengrot caused by the annual Christmas market. Temperatures during the Christmas market were considerably higher than before and after the event and we expected this to create a considerable disturbance for bats hibernating in the cave. We did not collect data on changes in humidity and sound levels, so the actual nature of the disturbance and the relative importance of various potential stimuli remain unclear. However, we can safely assume that the presence of many thousands of people, lighting and electrical power units during the Christmas market also play a role and that the Christmas market is a major cause of disturbance to hibernating bats in, at least part, of the Fluweelengrot.

Our study shows that the numbers of whiskered/Brandt's bat and Geoffroy's bat in the Fluweelengrot declined in relation to other limestone caves in Limburg. This indicates that the Christmas market had a negative influence on these species. For three other species, pond bats, Daubenton's bats and Natterer's bats, there was no evidence of such a relationship. However, between 1998 and 2010, after the first Christmas market, these latter three species showed a shift towards the north western parts of the cave, away from the Christmas market area. Although we do not know the underlying causes of the distributional changes, these results suggest that a number of individuals of these species moved possibly to avoid the influence of the Christmas market.

For practical reasons, temperature samples after 1997 were restricted to one winter only. The conclusions that we can draw from this data are therefore limited and do not offer insights into the possible long-term effects of the annual temperature rise that takes place during the Christmas market. We recommend the collection of microclimatic data on a regular basis (e.g. every three to five years) to see if the microclimate of the cave is changing (e.g. becoming warmer), which in the long run may negatively affect its suitability as a bat hibernaculum.

We are aware of the limitations of our method for investigating the distribution of bats. The two-dimensional projection of the bats' distribution that we used is a simplification of the actual distribution, as bats also locate themselves at different heights. In our opinion, however, this method is sufficient for the conclusions that we have drawn here.

The population trends of whiskered/Brandt's bat and Geoffroy's bat in the Fluweelengrot may be related to other factors. However, we have no indications of other human activities (e.g. the number or pattern of visitors during the year) or other conditions in the Fluweelengrot that would influence hibernating bats having changed since 1980, except for the introduction of the Christmas market.

The Christmas market in the Fluweelengrot is a form of intensive commercial exploitation, which is not a unique phenomenon in the marl quarries in south Limburg. A similar Christmas market, with even more visitors, is organised annually in the nearby Gemeentegroeve. Like the Fluweelengrot, this cave is home to hibernating Geoffroy's, pond and greater mouse-eared bats, all Habitats Directive Annex II-species. The impact of this event on the bats in this quarry has not yet been studied. In several other quarries in Limburg the number and diversity of sporting and other recreational activities, including cave biking, quad riding, abseiling and archery have increased over the last few decades. Guided tours and marl carving have been taking place in the Fluweelengrot, since the 1940s (Sluiter & van Heerdt 1957), but the levels of intensity between 1980 and 2010 seems to be relatively unchanged. Compared to these activities, the Christmas market is a major source of disturbance. Furthermore, the Christmas market coincides with the bats' hibernation period, while activities that take place between spring and autumn have less effect. The fact that the Christmas market is set up after the bats have settled for hibernation, makes this an unpredictable source of disturbance, which the bats can not anticipate by choosing locations away from the market area.

In spite of the potential threats, increases have been observed in the population levels of several species of bats in quarries in south Limburg, at least since the early 1980s (e.g. Dijkstra et al. 2006, Verboom 2006, Grol & Voûte 2010). These trends do not just reflect the increasing number of hibernacula investigated over the years, but are, at least partly, thought to be real population increases (Dijkstra et al. 2006). There may be various species specific reasons for these positive trends. The banning of certain pesticides and wood preservatives, improvements in the conditions in hibernacula for bats, and even climate change may all have played a role (e.g. Limpens et al. 1997, Dijkstra et al. 2006). The effects of ceasing the practice of banding bats in their hibernacula, recognised as a major mortality factor and a cause of decline of several bat species (Sluiter et al. 1971, Daan 1980, Voûte et al. 1980, Daan et al. 1982, Baker et al. 2001, Dietz et al. 2006) is unlikely to play a role here. This was practised in the marl caves in Limburg from the 1930s on, but was abandoned in 1959. Thereafter, identification in hibernacula has been done without handling the bats. Some species appear to have benefited from these measures, enabling the numbers of hibernating bats to increase over the last decades. In the light of these developments, increasing human activity in a number of these bat hibernacula, and the intention of the provincial and local governments to further intensify tourism in the region, including in the quarries, is a cause of concern (e.g. ZKA Markt & Beleid & NIB Consult 2001).

Disturbance of bats in their hibernacula can affect bats in different ways. Arousals that occur in addition to natural arousals during a winter, e.g. as a consequence of human disturbance, increase fat depletion and reduce bats' chances of surviving the hibernation period. Johnson et al. (1998), for instance, found that Indiana bats (*Myotis sodalis*) in a hibernaculum with few visits lost less weight than those in a more frequently visited hibernaculum (average number of winter visits: 5.5 and 378 respectively). This supports the argument that arousals during the hibernation have a negative effect on the bats' condition, with possible consequences for their survival rates and reproduction success in the following spring (Boyles & Brack 2009).

A bat may respond to arousal from unnatural conditions in several ways. It may remain in the same location and hide deeper in its crevice, or it may move to another location, either in the same or in another hibernaculum. Our study showed a negative trend (compared to other quarries) in the populations of whiskered/Brandt's bat and Geoffroy's in the Fluweelengrot after 1997, but did not show a change in their distribution throughout the cave. On the other hand, while there were no discernible differences in the population levels of pond bat, Daubenton's bat and Natterer's bat, there was evidence of these species having moved in a north westerly direction in the quarry. These results suggest that some species of bats remained in the Fluweelengrot (albeit in different locations), while others moved to other hibernacula or found hiding places where they were invisible for the researchers.

One problem with surveys of hibernacula is that the relationship between the number of bats recorded and the actual number of bats present is not always clear. Punt & van Nieuwenhoven (1957), for instance, marked bats of different species (brown long-eared bat, lesser horseshoe bat *Rhinolophus hipposideros* and several *Myotis* species) in a quarry with radioactive bands, and found that between 20 and 45% of the bats were hidden in invisible locations. Bats in deep crevices may be overlooked, especially in complex sites like marl caves, where cracks and crevices can be deep and hard to inspect. It is possible that some of the bats in the Fluweelengrot moved deeper into cracks and crevices as a reaction to the Christmas market and were overlooked during the counts. This could explain the trends in the numbers of recorded whiskered/Brandt's and Daubenton's bats. For Geoffroy's bat this is not a very likely explanation, as this species does not usually hide in cracks and fissures, but prefers to hang freely on ceilings, often in a dome-shaped space (Bezem et al. 1964).

Displacements within a hibernaculum do occur throughout the winter season. Some bats are found in exactly the same location for several months, whereas some individuals are recorded at a certain locality on only one visit (Daan & Wichers 1968). Studies of bats hibernating in south Limburg's quarries suggest species specific differences in this behaviour. According to ter Horst & van Nieuwenhoven (1958), Geoffroy's bat has long and uninterrupted hibernation bouts of ten weeks or longer, while Daubenton's bat and Natterer´s bat may remain in torpor for periods of around eight weeks and whiskered/Brandt´s bats for six weeks. Other species were found to wake up more frequently. Presumably, bat species with more frequent periodical arousals also exhibit a higher displacement activity (Daan & Wichers 1968).

Hibernating bats are found in a range of different positions which offer different degrees of protection; from very exposed hanging positions to narrow crevices and holes. It is clear that the position influences exposure to microclimatic factors. Climatic adaptation is an important factor influencing the distribution of bats (Bezem et al. 1964, Daan & Wichers 1968). For some bats the temperature zone that they select within hibernacula is an important survival factor (Ransome 1968).

As yet, there is no consensus about the role and relative importance of different non-tactile stimuli in provoking bat arousals. De Wilde & van Nieuwenhoven (1954) tried to find which stimuli, caused by humans visiting a limestone quarry in south Limburg, bats in hibernation were sensitive to. They concluded that warm air rising from gasoline lamps always had an effect; rays of light pointed at the animals at a distance of about 30 cm caused arousal in some cases and the sounds of human voices never aroused hibernating bats. By contrast, laboratory experiments by Speakman et al. (1991) on six common British species indicated that, under experimental conditions, non-tactile disturbances only lead to arousals in few cases, and caused only minimal increases in energy expenditure. They suggested that it may not be necessary to prevent non-tactile disturbances; although they pointed out that their interpretation of responses to non-tactile stimuli may not apply in natural conditions. The results of Speakman et al. (1991) are contradicted by Thomas (1995), who argued that light and the sound of human voices provoked an increase of flight movements of little brown myotis and northern myotis (*Myotis septentrionalis*) in a mine. While only a small proportion of the bats responded directly to the non-tactile stimuli caused by humans, these arousals propagated through the hibernating bat population, leading to increased flight activity of some bats up to 8.5 hours after the human visits.

The results of our study stress the need to further investigate the possible impact of recreational and other commercial activities in the caves of south Limburg. Many of these are major hibernacula for bats, and may house hundreds of bats each winter, including important numbers of species protected under Annex II of the EU Habitats Directive, i.e. Geoffroy's bats, pond bats and greater mouse-eared bats. We propose that impact assessment studies should be carried out to investigate the potential effects of human activities on bats in the caves. Activities that take place during the winter period may in particular pose serious threats to hibernating bats in these important hibernacula.

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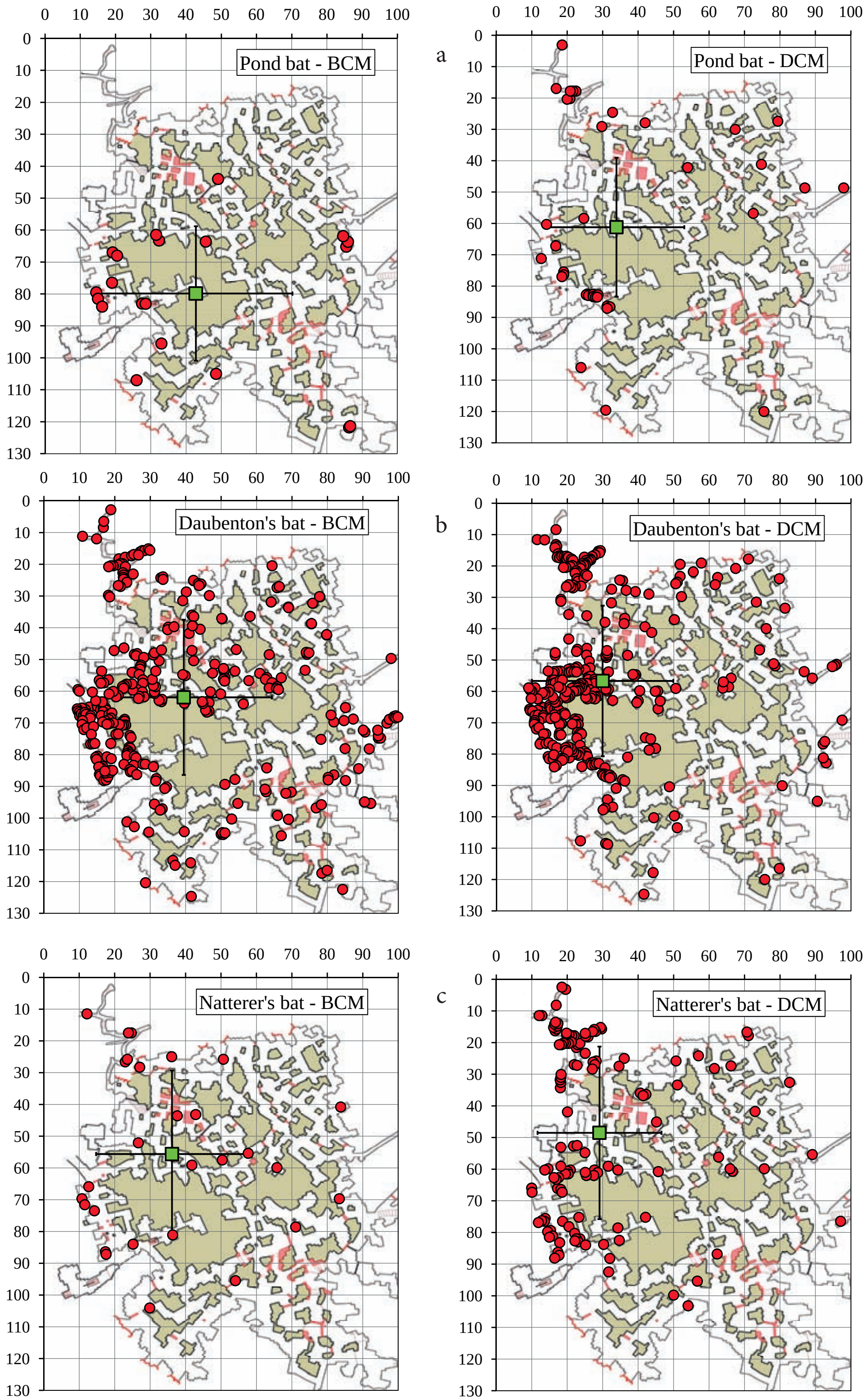
Pond bat - BCM
a
Pond bat - DCM
Daubenton's bat - BCM
b
Daubenton's bat - DCM
Natterer's bat - BCM
c
Natterer's bat - DCM
0 10 20 30 40 50 60 70 80 90 100
0 10 20 30 40 50 60 70 80 90 100 110 120 130

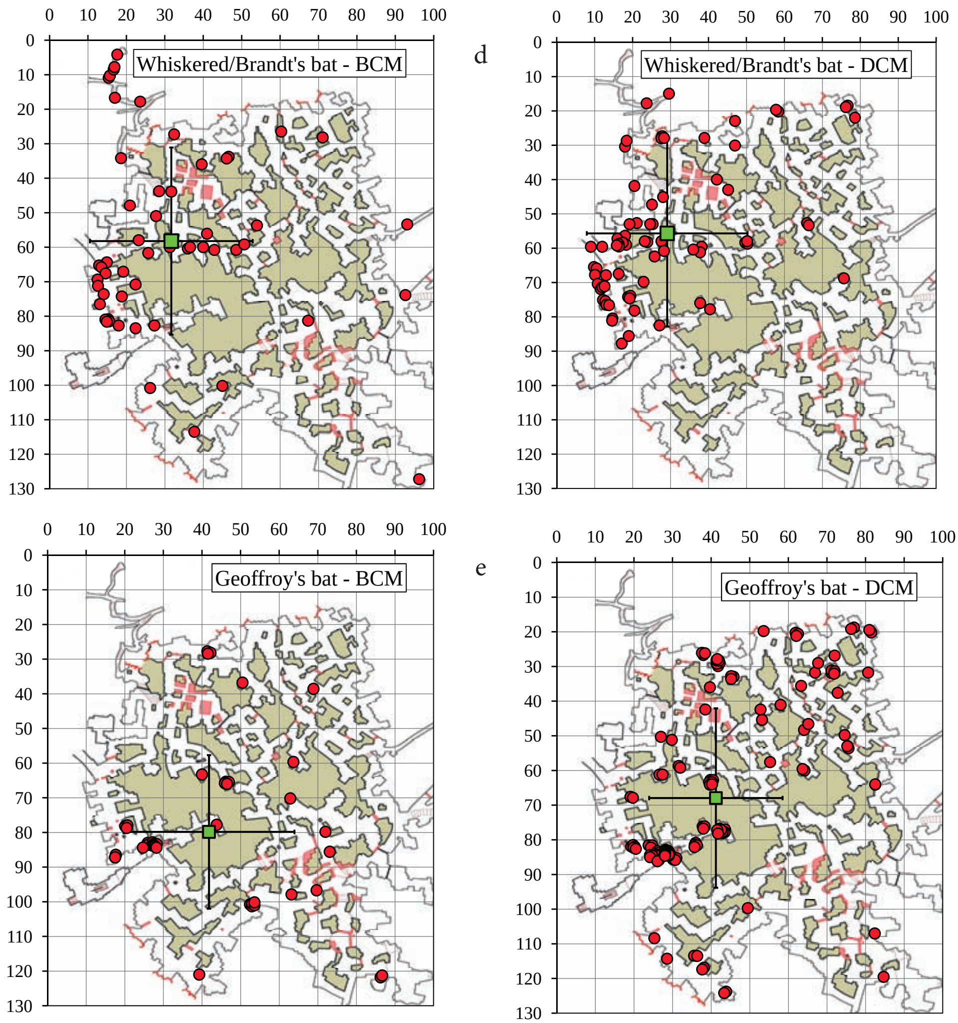

Figure 6. Map of the Fluweelengrot with the locations of pond bats (a), Daubenton's bats (b), Natterer's bats (c), whiskered/Brandt's bats (d) and Geoffroy's bats (e) in the period 1990-1997, here referred to as BCM (left), and DCM (1998-2010) (right) periods, with mean (green square) and standard deviation.

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Samenvatting

Effecten van een kerstmarkt op overwinterende vleermuizen in een mergelgroeve

Sinds november/december 1997 vindt in de Fluweelengrot, een mergelgroeve in de zuidlimburgse gemeente Valkenburg, jaarlijks een kerstmarkt plaats. Over een periode van vier tot vijf weken bezoeken elk jaar meer dan 100.000 mensen deze markt, die bestaat uit circa 50 stalletjes die zijn opgesteld langs een route door de groeve. Uit metingen in de winter van 2009-2010 bleek dat de temperatuur tijdens de kerstmarkt in een deel van de groeve 5-8 °C hoger lag (tot maximaal 20,1 °C) dan ruim een maand erna. We onderzochten de mogelijke effecten van deze potentiële bron van verstoring op de aantallen en verspreiding van vleermuizen in de groeve. In totaal werden in de onderzoeksperiode 1980-2010 tenminste zeven soorten vleermuizen aangetroffen. Vale vleermuizen (*Myotis myotis*) en grootoorvleermuizen (*Plecotus auritus/austriacus*) werden in zeer kleine aantallen aangetroffen. Voor de andere vijf soorten zijn trendanalyses gedaan. Hieruit bleek dat de trends van de baard-/Brandt's vleermuis (*M. mystacinus/Brandtii*) en de ingekorven vleermuis (*M. emarginatus*) over 1980-2010 significant achterbleven bij de gemiddelde trend van deze soorten in 89 andere Limburgse mergelgroeven. We onderzochten tevens de verspreiding van waarnemingen van genoemde vijf soorten over de Fluweelengrot. Drie soorten, de watervleermuis (*Myotis daubentonii*), de meervleermuis (*M. dasycneme*) en de franjestaart (*M. nattereri*), bleken na de eerste kerstmarkt in november/december 1997 significant meer in noordwestelijke richting te zijn waargenomen dan in de voorafgaande periode 1990-1997. Dit komt vooral door een toename van het aantal waarnemingen in een deel van de groeve buiten het kerstmarktgebied. De resultaten suggereren een negatieve invloed van de kerstmarkt op de vleermuizen in de groeve. Omdat we alleen temperatuur hebben gemeten, is niet bekend welke factoren (temperatuur, luchtvochtigheid, licht, geluid) een rol hebben gespeeld. Gezien het grote belang van de Limburgse mergelgroeven als winterverblijf voor vleermuizen, adviseren we om nader onderzoek te doen naar

de potentiële effecten van de toenemende toeristische druk op overwinterende vleermuizen in de groeven.

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